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EXAMINER	
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ART UNIT	PAPER NUMBER
1652	15

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/754,083	GREEN ET AL.	
	Examin r	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the c ver sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 February 2003* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-14 and 26-31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-14 and 26-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Claims 1-14, 26-31 are presently pending in this application.

Applicants' amendments and arguments filed on 2-14-03, paper No.14, have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claim Objections

Claims 2, 4, 7-8 and 13 are objected to because of the following informalities: Claims 2, 4, 7-8, 13 do not recite biological names of microorganisms in italics which is the norm while reciting biological names. However, applicants recite the yeast name in italics in claim 9. Appropriate correction is required.

Claim 10 is objected to because of the following informalities: Claim 10 recites that the heterologous gene is incorporated into the chromosome of the bacterium. However, it is well known in the art that bacteria do not have a chromosome *per se*, even though they have a single large DNA molecule which comprises the "genome" of the bacteria. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 1 and claims 2-13, 26-28 which depend from claim 1 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 recites the phrase "wherein the heterologous gene expression expresses an *active* pyruvate decarboxylase". The metes and bounds of the term "active" is not clear to the Examiner. Furthermore, the entire phrase appears to be redundant since such a recitation appears already in the claim.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3-4, 27-28 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a Gram-positive thermophilic bacterium transformed with pyruvate decarboxylase gene obtained from *Z.mobilis* or *S.cerevisiae*, --both of which are known to be heat tolerant and are active at higher temperatures at which thermophiles grow--, does not reasonably provide enablement for such a bacterium transformed with pyruvate decarboxylase isolated from any or all sources including mutants, variants and recombinants. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in In re Wands (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the

prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 3-4, 27-28 are so broad as to encompass bacterium transformed with pyruvate decarboxylase isolated from any or all sources. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of pyruvate decarboxylases broadly encompassed by the claims. Since it is well known in the art that thermophilic bacteria grow at an elevated temperature and invariably produce thermotolerant enzymes, predictability of which pyruvate decarboxylase available from the innumerable number of sources can be used requires a knowledge of and guidance with regard to which specific ones in the large group are tolerant and the detailed knowledge of the isolation, characterization and the cDNA clones of such pyruvate decarboxylase in order to transform any Gram-positive bacterium. Furthermore, since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification in terms of the decarboxylase activity), and detailed knowledge of the ways in which the proteins' structure relates to its function for those skilled in the art contemplating on using variants of pyruvate decarboxylase. However, in this case the disclosure is limited to the Gram-positive bacterium transformed with only two thermotolerant pyruvate decarboxylase obtained either from *Z.mobilis* or *S.cerevisiae*.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompasses thermophilic bacterium transformed with all or any pyruvate decarboxylase including variants, mutants and recombinants because the specification does not establish: (A)regions of the protein structure which may be modified without effecting decarboxylase activity; (B) the general tolerance of all or any pyruvate decarboxylase such that it can be used in a thermophilic bacterium or tolerance of any or all pyruvate decarboxylase to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any pyruvate decarboxylase with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including Gram-positive thermophilic bacteria transformed with any pyruvate decarboxylase. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance,

determination of pyruvate decarboxylase genes having the desired biological characteristics (for use in a thermophile) is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 3, 4, 27, 28 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 3, 4, 27-28 are directed to thermophilic Gram positive bacterium transformed with a heterologous gene encoding pyruvate decarboxylase. Claims 3, 4, 27-28 are rejected under this section of 35 USC 112 because the claims are directed to bacteria transformed with a genus of polypeptides including modified polypeptide sequences, modified by at least one of deletion, addition, insertion and substitution of an amino acid residue that have not been disclosed in the specification. No description has been provided of all the polypeptide sequences encompassed by the claim. No information, beyond the characterization of the function has been provided by applicants which would indicate that they had possession of the claimed genus of modified polypeptides. The specification does not contain any disclosure of the structure of all the polypeptide sequences, including fragments and variants within the scope of the claimed genus. The genus of polypeptides claimed is a large variable genus including peptides which can have a wide variety of structures. Therefore many structurally unrelated polypeptides are encompassed within the scope of these claims. The specification discloses only

a single species of the claimed genus which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the claimed genus. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to previous Office action, applicants have traversed the above rejection. Applicants have now amended claim 1 by deleting the phrase "functional equivalents thereof". Applicants also provide a definition for the term "heterologous" from the dictionary and provide a list of other pyruvate decarboxylase available in the art, both of which were unnecessary. Examiner is well aware of the term "heterologous" and its meaning in the context of the claim. Applicants continue to claim a thermophilic bacterium transformed with any pyruvate decarboxylase without providing the structure and therefore, above claims still fail to comply with the written description requirements as explained in the above rejection. While there are many cDNAs available for pyruvate decarboxylase not all or any of them can be used to transform a thermophilic bacteria as explained above. Applicants also refer to the case *Enzo Biochem v. Gen-Probe Inc.* and argue that a reference in the specification to a deposit constitutes an adequate description of the deposited material sufficient to comply with the written description requirement. Without comparing the full prosecution histories of the above court case and the instant application it is not clear to the Examiner as to how the outcome of the above can be applied to the instant application. However, as claim 14 is no longer included in

the above rejection such arguments are moot. Examiner has withdrawn the rejection as previously applied but continues to maintain the above rejection as it applies to the above claims 3, 4, 27, 28.

Claims 12-14, 27-28, 30-31 are rejected because the invention appears to employ novel vectors and novel strains of microorganisms. Since the vectors and microorganisms are essential to the claimed invention, they must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. The claimed plasmids' sequences or the microorganisms are not fully disclosed, nor have all the sequences required for their construction been shown to be publicly known and freely available. The enablement requirements of 35 U.S.C. § 112 may be satisfied by a deposit of the plasmids/microorganisms. The specification does not disclose a repeatable process to obtain the vectors/microorganisms and it is not apparent if the DNA sequences are readily available to the public. Accordingly, it is deemed that a deposit of the plasmid and microorganisms should have been made in accordance with 37 CFR 1.801-1.809.

It is noted that applicants have deposited the plasmid and the microorganisms but there is no indication in the specification as to public availability. As the deposit has been made under the terms of the Budapest Treaty, an affidavit or declaration by applicants, or a statement by an attorney of record over his or her signature and registration number, stating that the specific strain has been deposited under the Budapest Treaty and that the strain will be irrevocably and without restriction or condition released to the public upon the issuance of the patent, would satisfy the deposit requirement made herein.

In response to the above rejection, applicants have traversed the above rejection arguing that the specification discloses that plasmid pFC1 was formed from a fusion of pAB124 and pUC18 and that the plasmid restriction enzyme cleavage map has been shown in Figure 8. Applicants argue that the plasmid pAB124 was known and used at the time the application was filed and that Bingham et al. teaches at Figure 2, a restriction map of the plasmid pBST22-zym by routine experimentation and therefore one of skill in the art would also have been able to make and use bacterial strain LN-DP1 without undue experimentation. Examiner respectfully disagrees with such an argument and reiterates that such an argument is not persuasive to overcome the above rejection. Bingham et al. do not provide the full sequence of the isolated plasmid or provide information regarding a biological deposit or declare that it is publicly available. Bingham et al. do not teach the specific bacterial strains *B.subtilis* or *B.stearothermophilus* TB124 from which they isolated the plasmid as publicly available either. Providing a reference in which the bacterial strain was used or the plasmid was used does not satisfy the biological deposit requirement unless such a reference declares the public availability of said biological materials. Therefore, Examiner continues to maintain the above rejection.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-4, 10-11, are rejected under 35 U.S.C. 103(a) as being unpatentable over Danilevich et al. (Translation of *Molekulyarnaya Biologiya*, 1994, Vol. 28(1):158-166), and Guagliardi et al. (Int. J. Biochem. Cell Biol., 1996, Vol. 28(2):239-246). Claims 1-4, 10-11 in this instant application are drawn to a Gram positive bacterium having native alcohol dehydrogenase activity that has been transformed with a heterologous gene encoding pyruvate decarboxylase, wherein the bacterium is a *Bacillus sp.*, selected from a group, comprising *B.stearothermophilus*, wherein the heterologous gene is incorporated into the chromosome of the bacterium or the Gram positive bacterium has been transformed with a plasmid comprising the heterologous gene.

Danilevich et al. teach Gram positive bacterium belonging to the *Bacillus sp.* which has been transformed with a plasmid comprising the heterologous gene from *Z.mobilis* encoding pyruvate decarboxylase and suggest the use of such strains for alcohol production. However, as the *Bacillus sp.* in the reference does not have native alcohol dehydrogenase activity Danilevich et al. teach the transformation of the same *Bacillus* with additional gene, alcohol dehydrogenase, required for the synthesis of alcohol. The above reference also teaches that introduction of two actively expressed genes, pdk and adh into *B.subtilis* while resulting in ethanol production may also alter the cell metabolism in an unpredictable manner. Therefore, the *Bacillus* sp. in the above reference while identical to the above invention in most aspects, differs from the invention in that the transformed bacteria does not have endogenous alcohol dehydrogenase activity.

Guagliardi et al. teach that *B.stearothermophilus*, a Gram-positive *Bacillus* has inherent alcohol dehydrogenase activity. The reference also teaches that the strain can grow at 70° C.

With the above two references in hand it would have been obvious to one of ordinary skill in the art, to use the strain taught by Guagliardi et al. in place of the *Bacillus* strain used by Danilevich et al. One of ordinary skill in the art would have been motivated to do so in order to overcome any unpredictable alteration of the metabolism of the transformed cell as taught by Danilevich and also lessen the complication of transforming the bacterium with two heterologous genes as opposed to only one. One of ordinary skill in the art would have a reasonable expectation of success since Danilevich et al. teach method of transforming a *Bacillus* with pyruvate decarboxylase from *Z.mobilis* and Guagliardi et al. teach the availability of a *Bacillus* which has inherent alcohol dehydrogenase activity.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

In view of the claim amendments and arguments presented by the applicants, Examiner has withdrawn the previous rejection of claims under 35 U.S.C. 102(b). However, claims 1-4 and 10-11 are now rejected under 35 U.S.C. 103(a) as obvious. Therefore, applicants arguments against previous rejection as claims being anticipated are moot.

Claims 1-8, 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Danilevich et al. (Translation of *Molekulyarnaya Biologiya*, 1994, Vol. 28(1):158-166), Hartley et al. (Biotechnol., 1983, pages :895-905), and the common knowledge in the art regarding the *Z.mobilis* pyruvate decarboxylase (for e.g. see Conway et al., 1987, J. Bacteriol., Vol. 169(3):949-954). Claims 1-8, 10-11 in this instant application are drawn to a Gram positive bacterium which has been transformed with a heterologous gene encoding pyruvate

decarboxylase, but has native alcohol dehydrogenase activity, wherein the bacterium is a thermophile, a *Bacillus sp.*, selected from a group comprising *B.stearothermophilus*, wherein the gene encoding lactate dehydrogenase has been inactivated by homologous recombination, wherein the heterologous gene is from *Zymomonas sp.* such as *Z.mobilis* or *S.cerevisiae* and wherein the bacterium has been transformed with a plasmid comprising the heterologous gene.

Danilevich et al. teach Gram positive bacterium belonging to the *Bacillus sp.* which has been transformed with a plasmid comprising the heterologous gene from *Z.mobilis* encoding pyruvate decarboxylase and suggest the use of such strains for alcohol production. However, as the *Bacillus sp.* in the reference does not have native alcohol dehydrogenase activity Danilevich et al. teach the transformation of the same *Bacillus* with additional gene, alcohol dehydrogenase, required for the synthesis of alcohol. As can be seen, the reference does not teach the use of a thermophilic strain such as *B.stearothermophilus* in place of *B.circulans* and does not teach that the lactate dehydrogenase be inactivated or that the heterologous gene be operatively linked to the LDH promoter from a *Bacillus* strain.

Hartley et al. teach the advantages of using a thermophilic microorganism such as *B.stearothermophilus* for production of ethanol. The reference teaches a "metabolic steering" strategy of developing strains of *B.stearothermophilus* for production of ethanol. The reference teaches that knocking out (i.e., inactivating) lactate dehydrogenase would steer the metabolic pathway of the above microorganism for production of more ethanol, as this is the enzyme that steers the aerobic pathway towards production of lactic acid rather than acetate and ethanol. The reference also teaches that one of skilled in the art can use such strains in a recombinant approach by introduction of a gene for pyruvate decarboxylase from yeast or *Zymomonas*. The

reference provides methods to select mutants that are lactate non-producers or low lactate producers in which the lactate dehydrogenase is inactivated.

Therefore, with the reference of Danilevich et al. which teaches the introduction of *Z.mobilis* pyruvate decarboxylase into *B.subtilis* and the reference of Hartley et al. which teaches the advantages of using a thermophilic *Bacillus* producing native adh and which has been genetically modified for alcohol production, it would have been obvious to one of ordinary skill in the art to transform *B.stearothermophilus* instead of *B.subtilis* as taught by Danilevich et al. and develop a strain for production of ethanol. One of ordinary skill in the art would have been motivated to do so as Hartley et al. teach that use of thermophilic bacteria for production of ethanol has certain economic advantages over other fermentation methods, provide thermophilic *Bacillus* strain producing native *adh* and in which the *ldh* gene has been inactivated and suggest the use of such strains in a recombinant approach of transforming the thermophilic strain with the *Zymomonas* pyruvate decarboxylase. One of ordinary skill in the art would have a reasonable expectation of success since Danilevich et al. demonstrate the transformation of another *Bacillus sp.* with the pyruvate decarboxylase of *Z.mobilis* and provide methods for performing such transformation. One of ordinary skill in the art would have a reasonable expectation of success because of the knowledge prevailing in the art that *Zymomonas* pyruvate decarboxylase is heat stable (see Conway et al.) and hence would be suitable for transforming a thermophilic bacteria which incidentally also has native alcohol dehydrogenase activity.

Therefore the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art.

In response to the previous Office action, applicants have traversed the above rejection arguing Examiner has not met with the "reasonable expectation of success" requirement for the obviousness rejection. Applicants mainly argue that Danilevich's *B.subtilis* transformed with *Z.mobilis* pyruvate decarboxylase does not have native *adh* activity and while Hartley et al. teach the manipulation of the genome of *B.stearothermophilus*, a Gram-positive bacterium that has native *adh* activity to inactivate the lactate dehydrogenase gene, one of ordinary skill in the art, however, would not have combined these teachings to arrive at the instant invention. This is because Danilevich et al. teaches that introduction of two actively expressed genes may alter the cell metabolism in an unpredictable manner. Applicants argue that because of the above reason, one of ordinary skill in the art would not have a reasonably expectation of success either. Furthermore, Applicants continue to argue that Hartley et al. does not remedy the deficiencies of Danilevich as the reference teaches that one might "try" a recombinant approach and also does not teach or suggest that a bacterium with native *adh* activity and expressing a heterologous pyruvate decarboxylase could be successfully produced and in fact, even if one of ordinary skill in the art looked to Danilevich for an expression vector encoding a pyruvate decarboxylase gene, she would have been taught that introduction of above two genes into a Gram-positive bacterium could alter its metabolism and would not reasonably have expected success. Examiner respectfully disagrees with such an argument. While Danilevich's statement that introduction of two actively expressed genes may alter the cell metabolism in an unpredictable manner may apply for *B.subtilis*, using that same argument to overcome the instant rejection is highly misplaced. This is because *B.subtilis* is not a thermophile and the above rejection does not involve transformation of the bacteria with two cDNAs as is the case in Danilevich et al.

reference. Examiner has also rewritten the rejection in order to overcome such arguments by the applicant.

Applicants continue their argument that contrary to what was expected from the teachings of the cited art (Danilevich's conclusion of unpredictable metabolism), the claimed bacteria have unexpected properties of a significant increase in ethanol production and superior growth characteristics. Examiner respectfully disagrees that such results were unexpected if any. This is because the expression (*to try*) in the Hartley's journal publication cannot be interpreted in the same manner as interpreted during the prosecution of a patent claim. Therefore, contrary to applicant's argument Hartley et al. actually suggest the above invention. Examiner respectfully disagrees with applicant's observation regarding the high yield of ethanol and growth of the transformant as unexpected because there are many teachings in the art (including the above two references used in the above rejection) which teach that recombinant bacteria transformed with *Z.mobilis* pyruvate decarboxylase will invariably tend to yield higher amounts of ethanol and while the metabolism of Danilevich's transformants may have been unpredictable. Such results cannot be extended to thermophilic organisms transformed with a single pyruvate decarboxylase gene as in the instant case. The unpredictable metabolism observed by Danilevich et al. may be restricted only to *B.subtilis* or occurs when two expressible cDNAs are used to transform a single *Bacillus*.

Next, applicants argue that one of ordinary skill in the art would have expected even less success at producing the bacterium of dependent claim 3 directed to a thermophilic Gram-positive bacterium which is transformed with a heterologous gene encoding pyruvate decarboxylase that is expressed and active and which has native alcohol dehydrogenase activity.

Applicants argue this is because, Hartley et al. teach that a suitable vector for expression of the pyruvate decarboxylase was not available and even if it were available a bacterium with such a vector may not gain the pyruvate decarboxylase function: "This strategy depends on developing suitable vectors for the thermophile and might fail if the enzyme were insufficiently thermostable". Examiner respectfully disagrees with such an argument as being persuasive to overcome the above rejection. This is because, while Hartley et al. was not aware of the availability of the suitable vector or the thermostability of the *Z.mobilis* pyruvate decarboxylase, there were teachings in the art of the suitable plasmids that could be used in thermophilic bacteria (for e.g. see Bingham et al. (J. Gen. Microbiol., 1980, Vol. 119:109-115) and that the pyruvate decarboxylase isolated from *Z.mobilis* was thermostable (for e.g. see Conway et al., 1987, J. Bacteriol., Vol. 169(3):949-954). Therefore, contrary to applicants argument it would have been well within the knowledge of those skilled in the art to select an appropriate vector for a thermophile and to use the pyruvate decarboxylase gene of *Z.mobilis* or *S.cerevisiae* which were well known to be thermotolerant. Because of the above reasons applicants argument that Danilevich et al. vector was tested on non-thermophilic bacteria and Hartley's caveat regarding the suitability of the vectors and function of pyruvate decarboxylase in a thermophile are not persuasive to overcome the above rejection. Furthermore, as Examiner has rewritten the rejection such arguments are also rendered moot. Therefore, the above rejection is maintained.

Claims 9-10, 26, 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Hartley et al. (Biotech., 1983, pages 895-905), and the common knowledge in the art that the yeast pyruvate decarboxylase in general are thermostable (for e.g. see Li H et al. (Biochemistry,

1999, Vol. 38:10004-10012). Claims 9-10, 26, 29 in this instant application are drawn to a Gram positive bacterium which has been transformed with a heterologous gene encoding pyruvate decarboxylase, but has native alcohol dehydrogenase activity, wherein the heterologous gene is incorporated into the chromosome (genome) of the bacterium and wherein the bacterium is a thermophile and comprises inactivated lactate dehydrogenase gene.

Hartley et al. teach the advantages of using a thermophilic microorganism such as *B.stearothermophilus* which has native alcohol dehydrogenase activity for production of ethanol. The reference teaches a "metabolic steering" strategy of developing strains of *B.stearothermophilus* for production of ethanol. The reference teaches that knocking out (i.e., inactivating) lactate dehydrogenase would steer the metabolic pathway of the above microorganism for production of more ethanol, as this is the enzyme that steers the aerobic pathway towards production of lactic acid rather than acetate and ethanol. The reference also teaches that one of skilled in the art can use such strains for introduction of a gene for pyruvate decarboxylase from yeast or *Zymomonas*. The reference provides methods to select mutants that are lactate non-producers or low lactate producers in which the lactate dehydrogenase is inactivated.

Li et al. teach the cDNA clone of yeast pyruvate decarboxylase and also teach that the yeast decarboxylase is thermotolerant enzyme (see the entire article, specifically figure 4). Combining the teachings of the above two references it would have been obvious to one of ordinary skill in the art to transform the *B.stearothermophilus* with a plasmid comprising the wild type yeast pyruvate decarboxylase gene taught by Li et al. While Li et al. do not specifically state that the pyruvate decarboxylase gene in their reference is yeast pdc5, Examiner takes the

position that the yeast decarboxylase gene is indeed yeast pdc5. Since the Office does not have the facilities for examining and comparing applicants' protein with the protein of the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product and the product of the prior art (i.e., that the protein of the prior art does not possess the same material structural and functional characteristics of the claimed protein). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and In re Fitzgerald et al., 205 USPQ 594. One of ordinary skill in the art would be motivated due to the suggestion by Hartley to take a recombinant route to make transformed *B.stearothermophilus* for ethanol production using either yeast or *Z.mobilis* genes. One of ordinary skill in the art would have a reasonable expectation of success since the art teaches methods of transformation, methods of incorporation of heterologous DNA into the bacterial genome and plasmids that are compatible with thermophilic bacteria and also due to the fact that the yeast pyruvate decarboxylase is thermotolerant.

Therefore the above invention would have been *prima facie* obvious to one of ordinary skill in the art.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(f) or (g) prior art under 35 U.S.C. 103(a).

Conclusion

None of the claims are allowable.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Manjunath Rao whose telephone number is (703) 306-5681. The Examiner can normally be reached on M-F from 7:30 a.m. to 4:00 p.m. If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, P.Achutamurthy, can be reached on (703) 308-3804. The fax number for Official Papers to Technology Center 1600 is (703) 305-3014. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

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